Molecules to Medicine
Molecular Biology Sub-Block

Tools of Molecular Biology I

Matthew Taliaferro, PhD

October 16, 2020

matthew.taliaferro@cuanschutz.edu

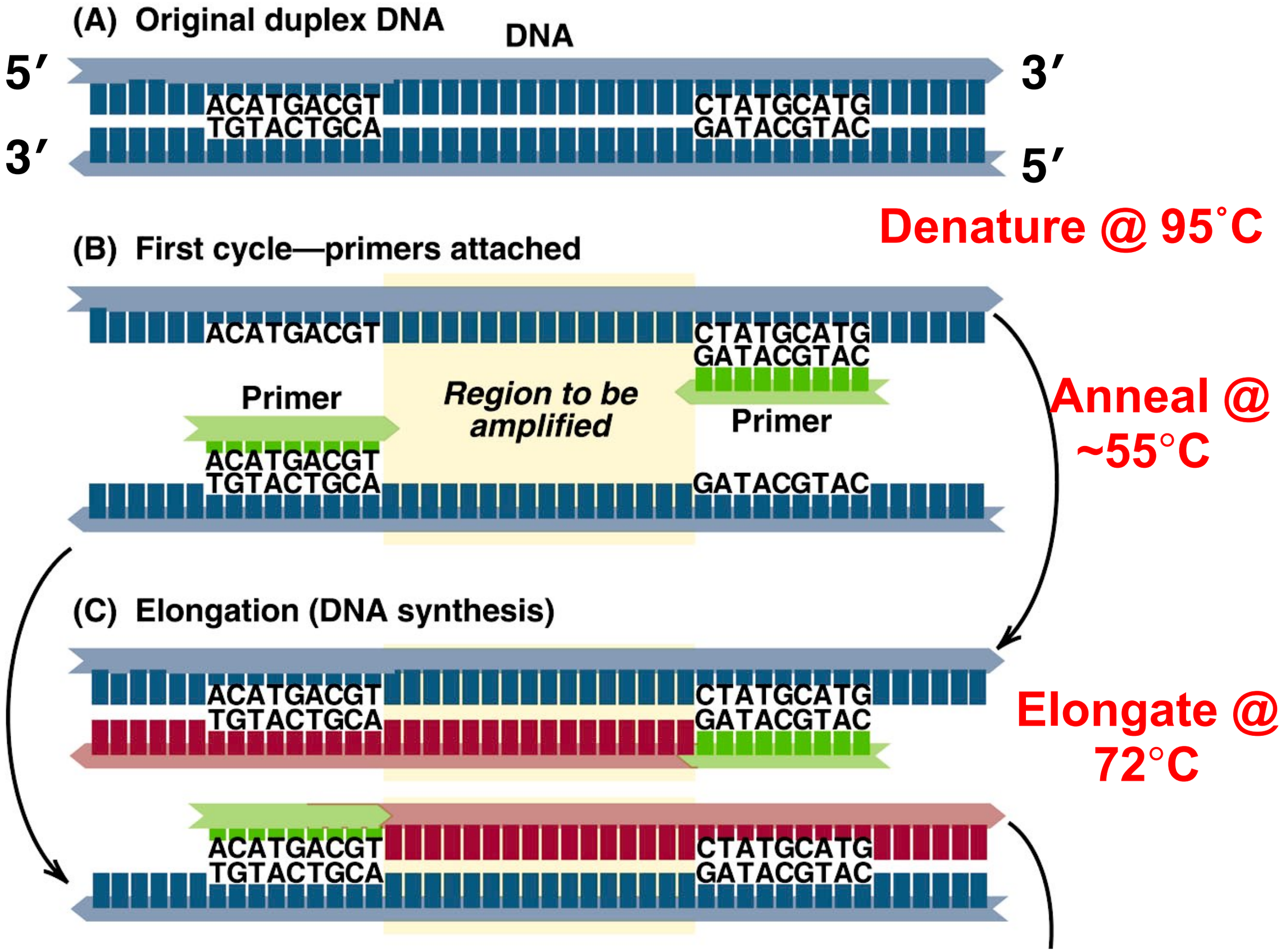
Objectives

1. Describe distinct uses of PCR amplification in the diagnosis of a genetic condition in patients.
2. Give an example of a disease that can be diagnosed using a restriction fragment length polymorphism (RFLP) and a use of DNA fingerprinting. Describe at least three experimental stages required in each of these procedures.
3. Describe the use of Variable Number Tandem Repeats (VNTRs) for genotyping and identification of a DNA sample.
4. Explain the principles of electrophoretic separation and analysis of DNA, RNA, and protein targets.
5. Describe the principles behind real time PCR and its application to the diagnosis or monitoring of infection.

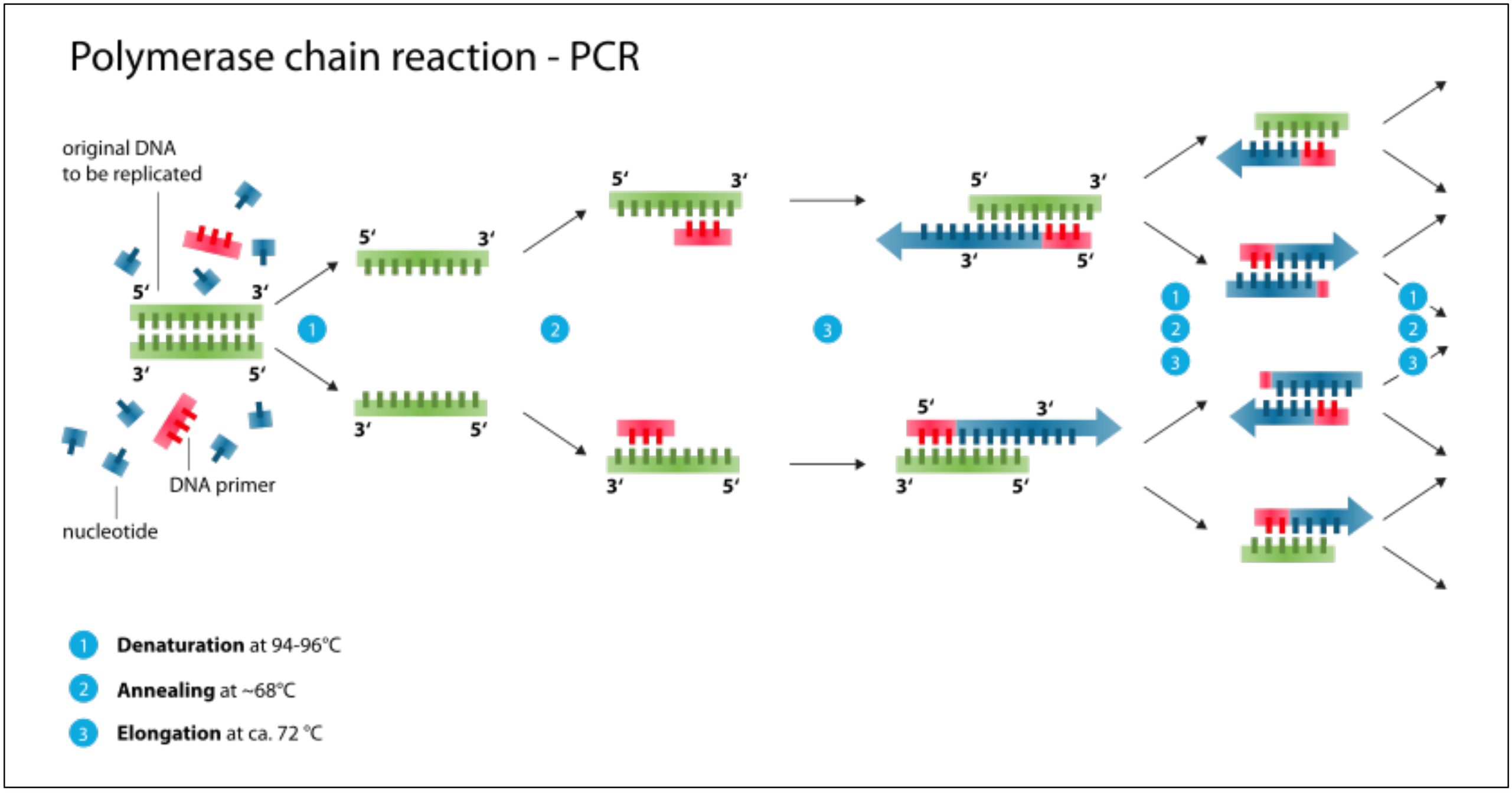
Outline

- **PCR and gel electrophoresis**
  - **Principles of nucleic acid amplification and analysis in biotechnology and medicine**
- **Application of PCR-based tools to human genetics**
  - **Repeat expansion PCR, DNA fingerprinting, RFLP, Sanger sequencing**
- **Application of PCR-based tools to gene expression quantification**
  - **Principles of quantitative PCR and its application to medicine**

Polymerase Chain Reaction



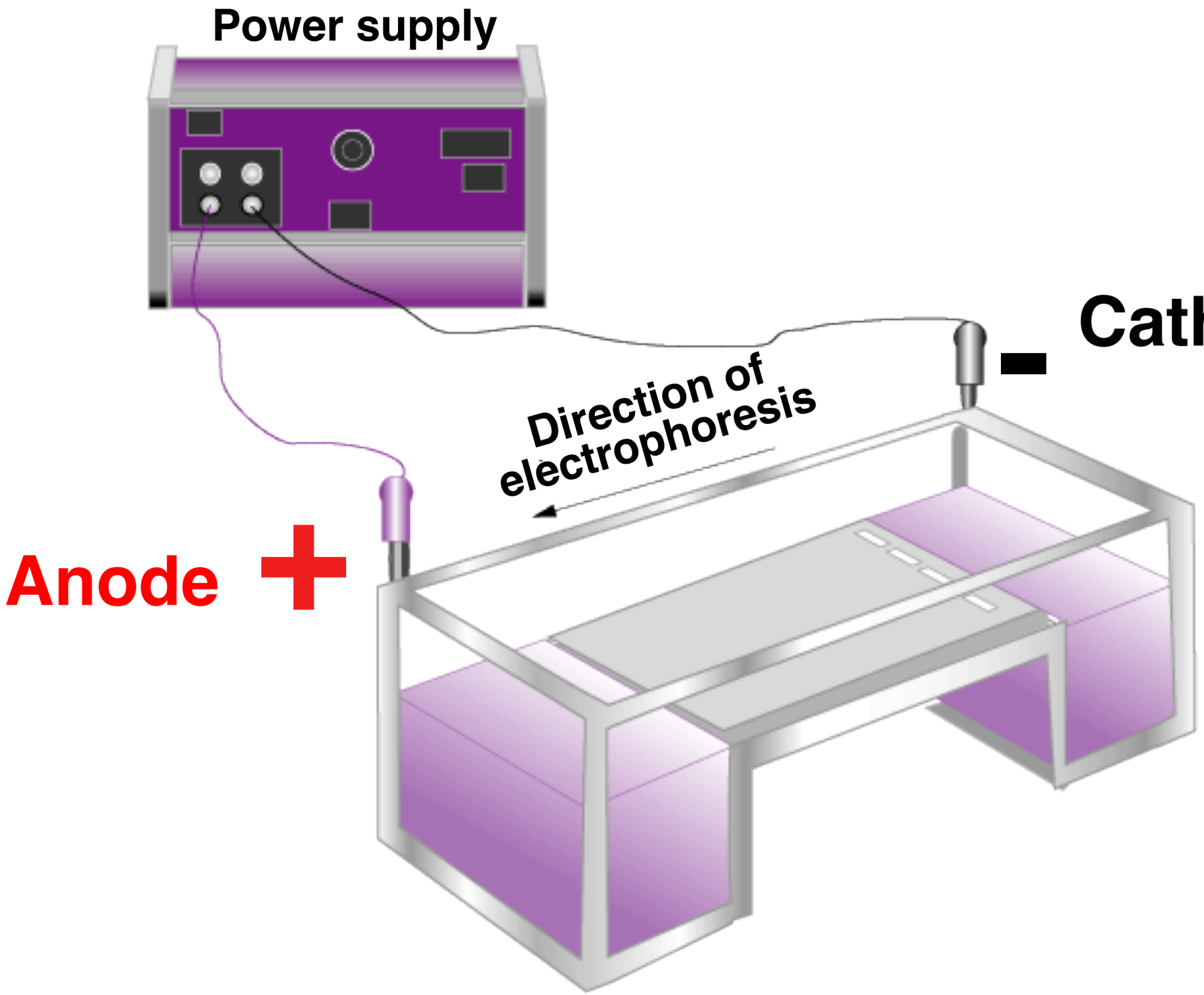
Polymerase Chain Reaction (cont.)



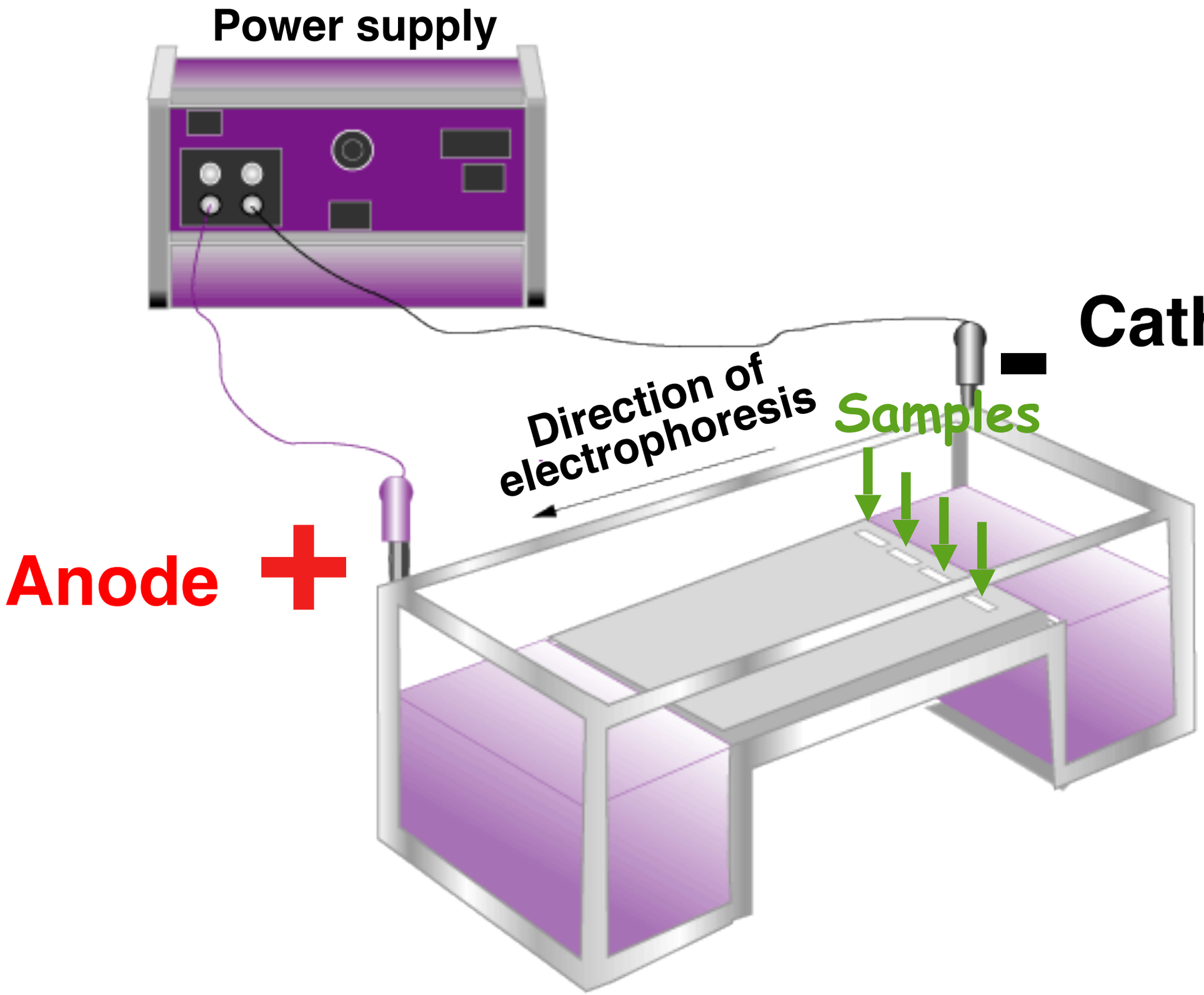
*number of DNA molecules doubles with each cycle... 2, 4, 8, 16, 32…

After 25 cycles have 2^{25} molecules of DNA from just one!

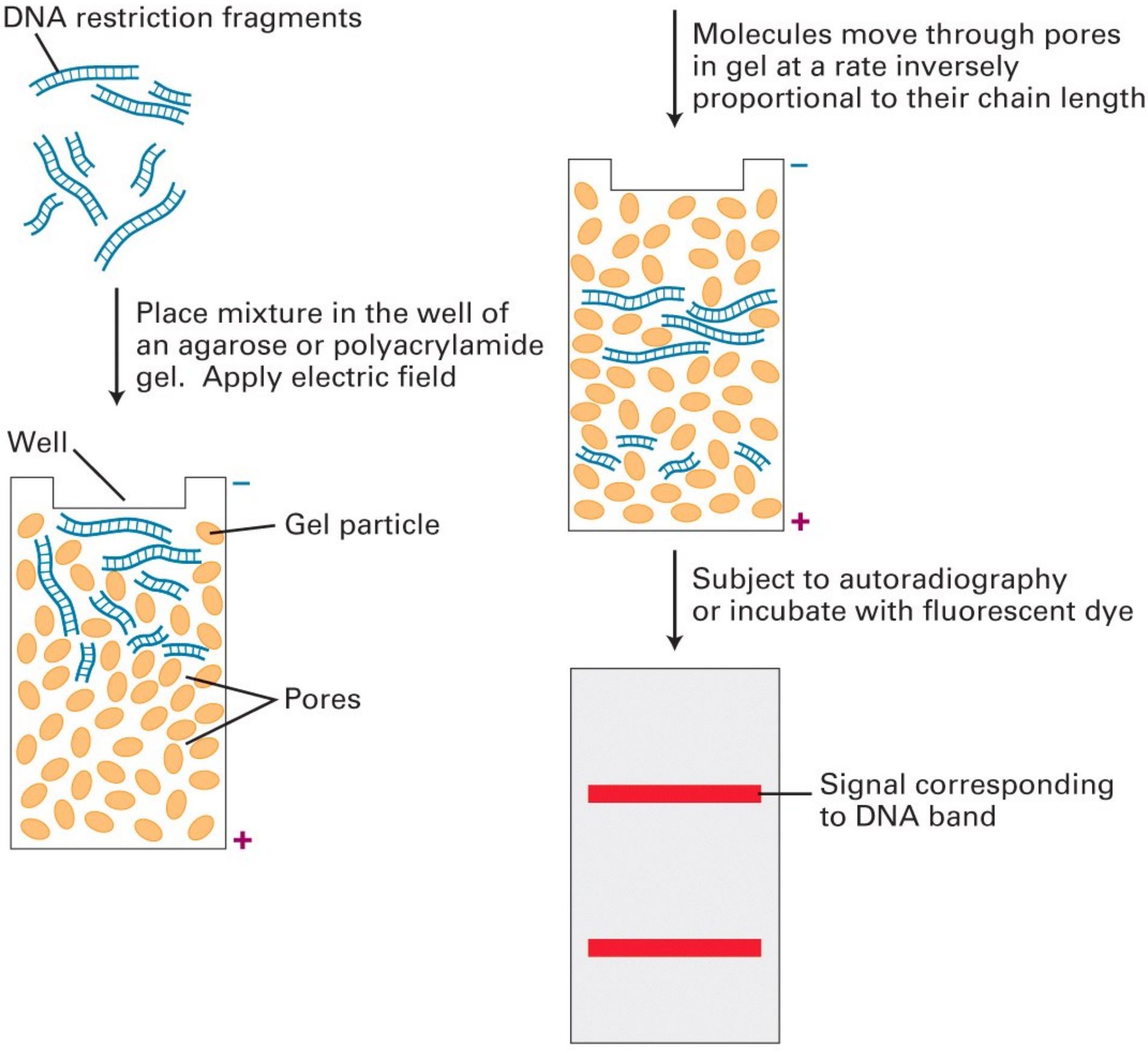
Gel electrophoresis for separation of DNA molecules



Gel electrophoresis for separation of DNA molecules

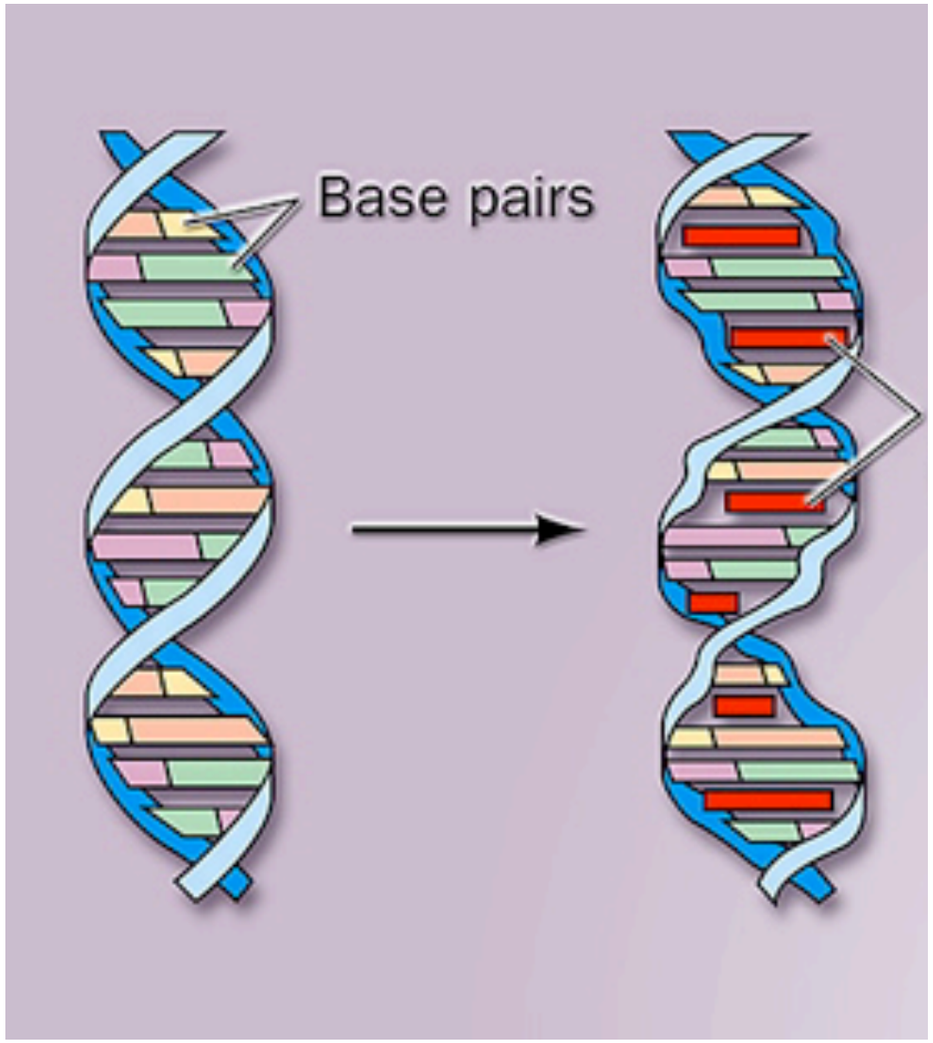


Gel electrophoresis separates DNA based on size



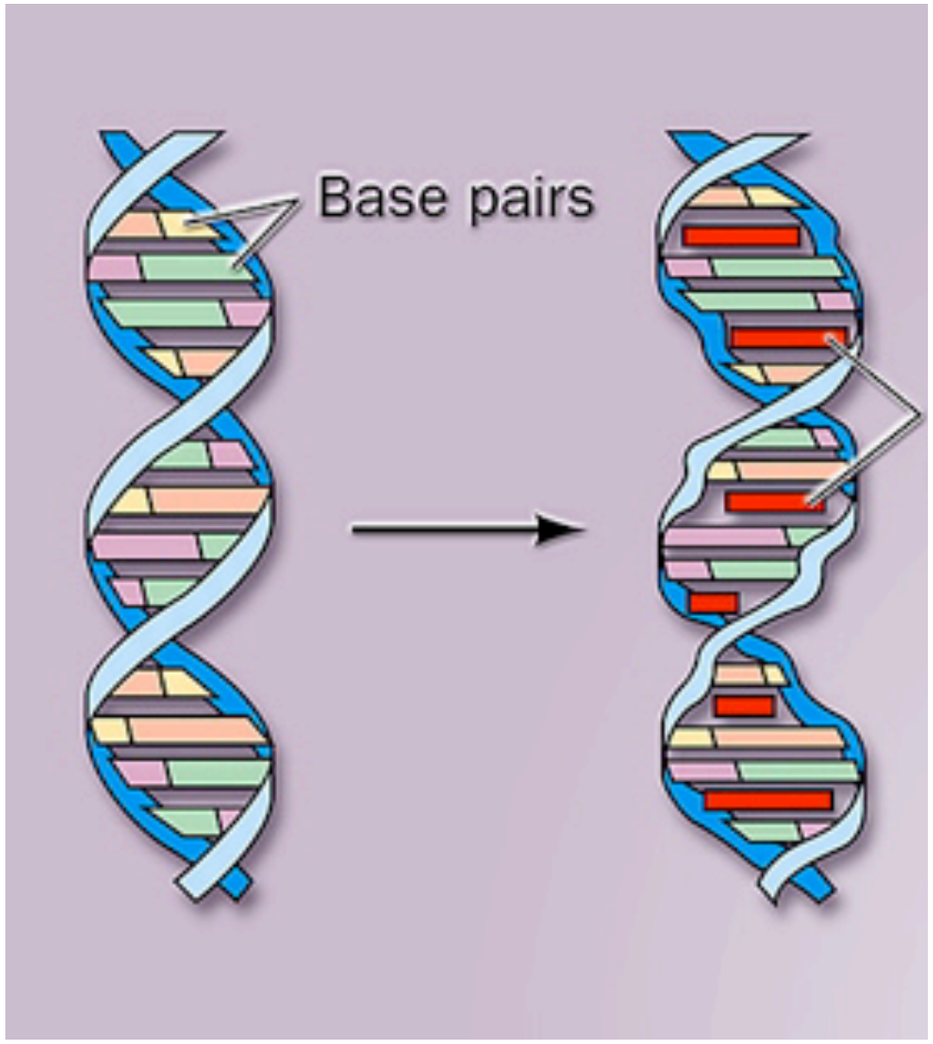
Visualizing DNA using intercalating dyes

Intercalating dye
e.g. ethidium
bromide

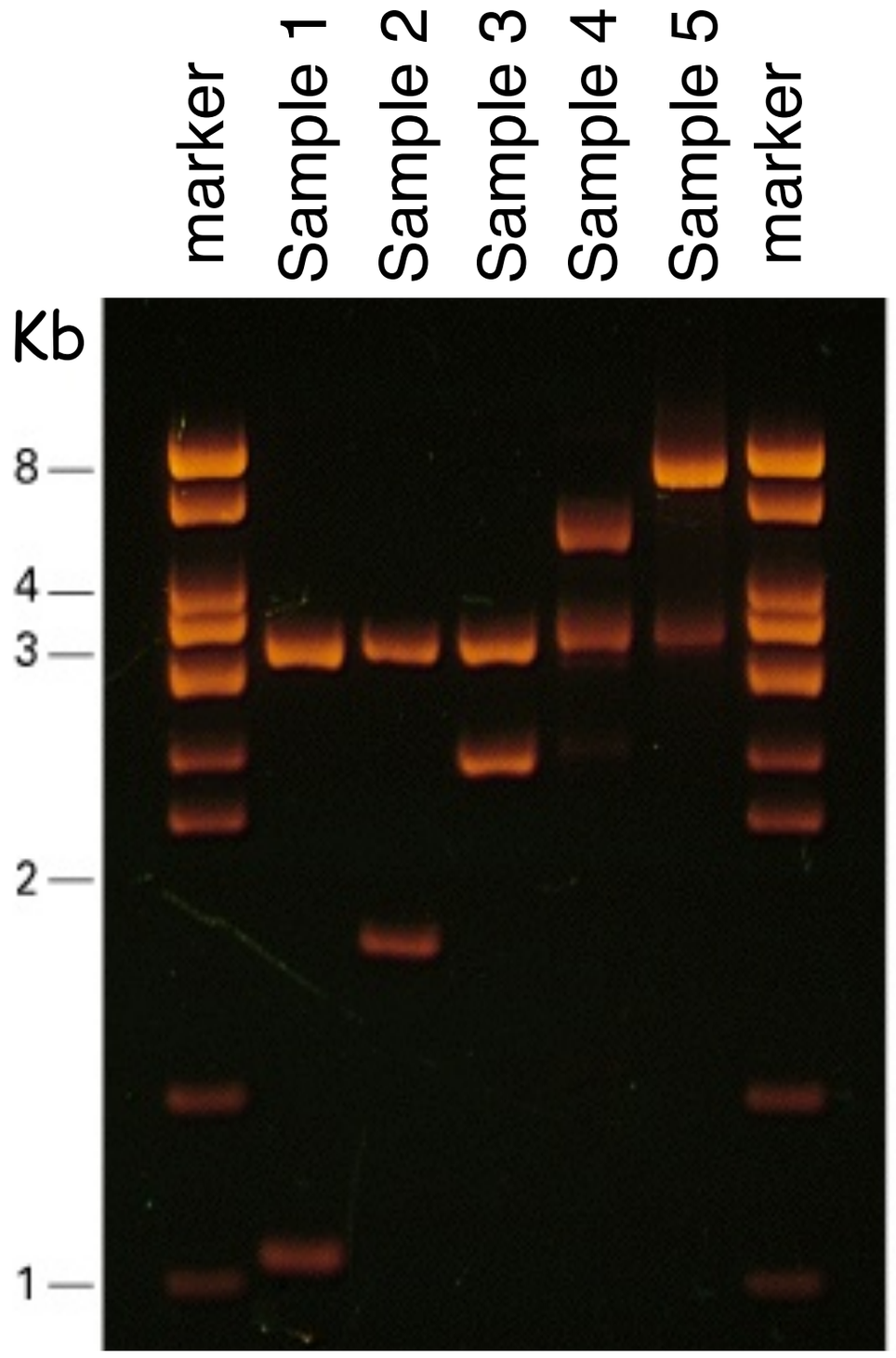


Visualizing DNA using intercalating dyes

Intercalating dye
e.g. ethidium
bromide



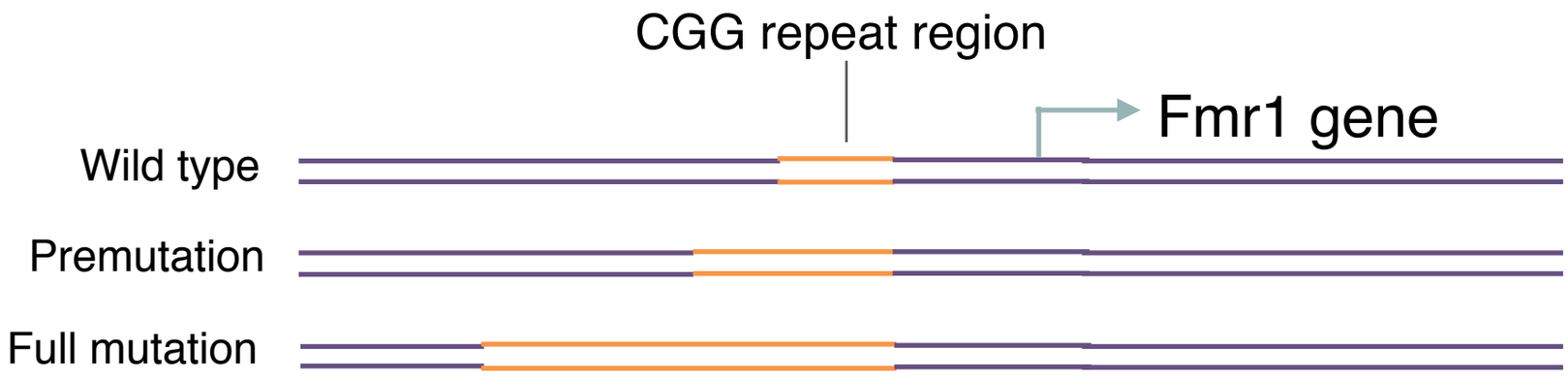
UV light



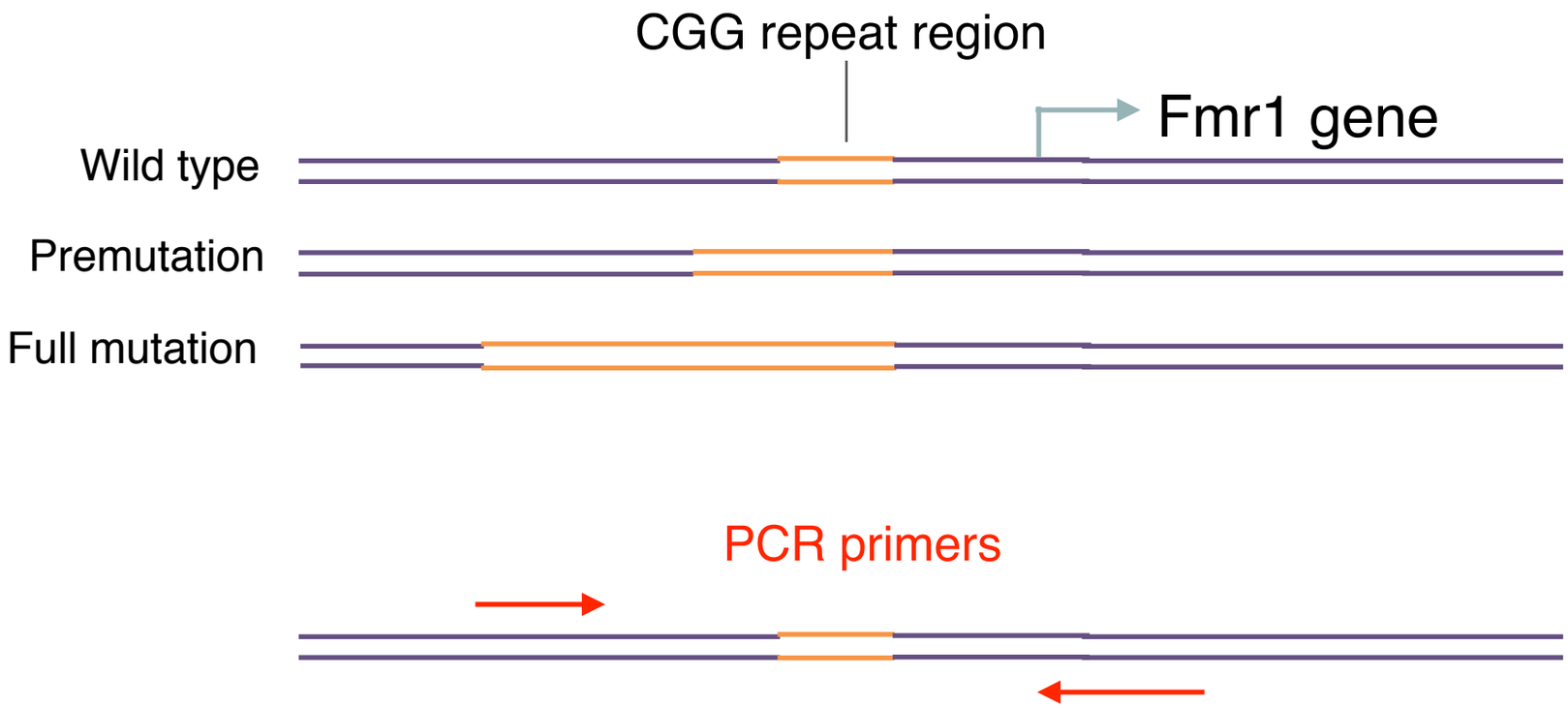
Tools for investigating DNA sequence changes between samples (patients)

1. Interrogation of repeat expansions using PCR
2. DNA fingerprinting
3. Restriction fragment length polymorphism
4. Sanger sequencing

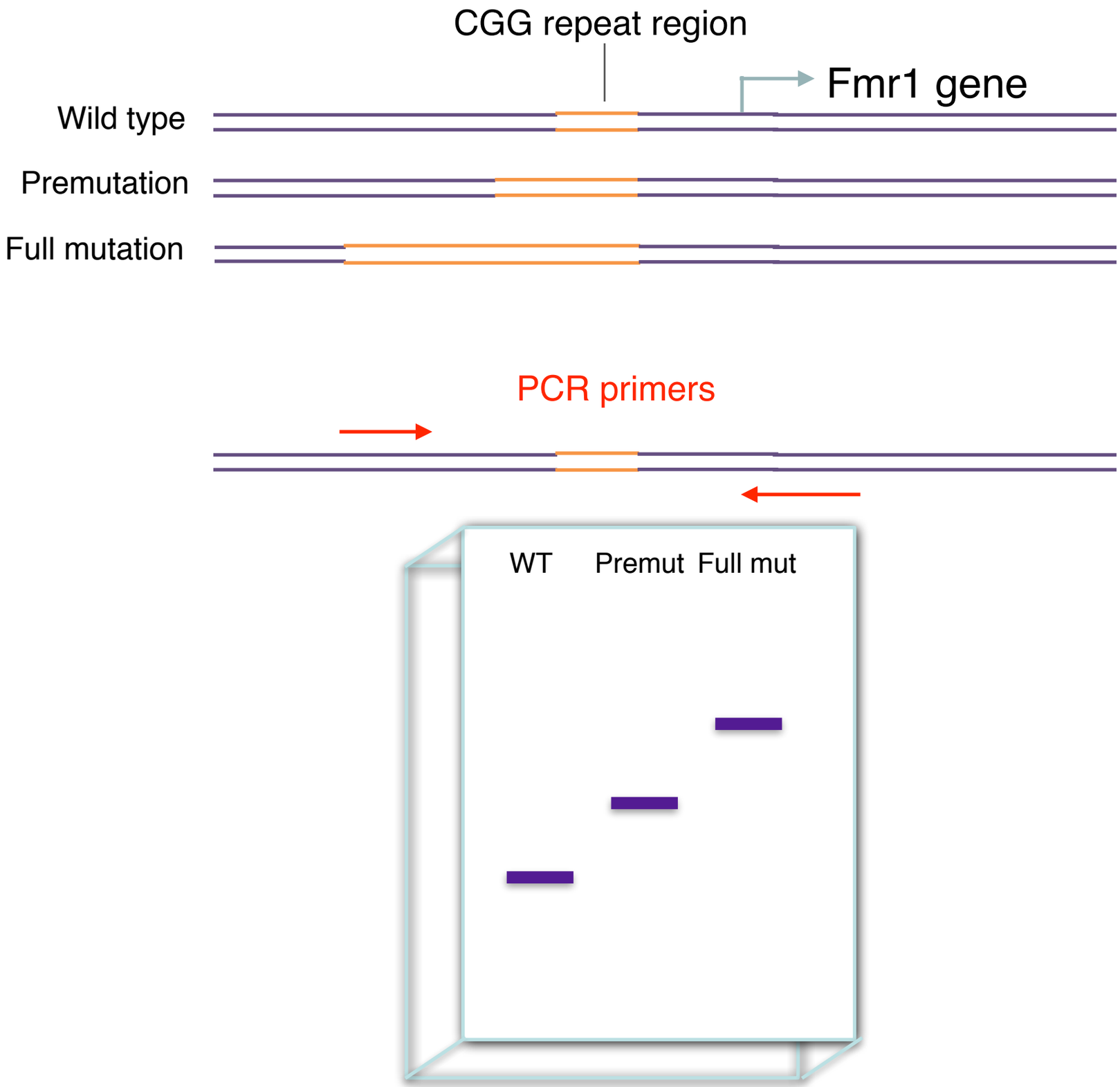
Detection of Trinucleotide Repeat Expansion



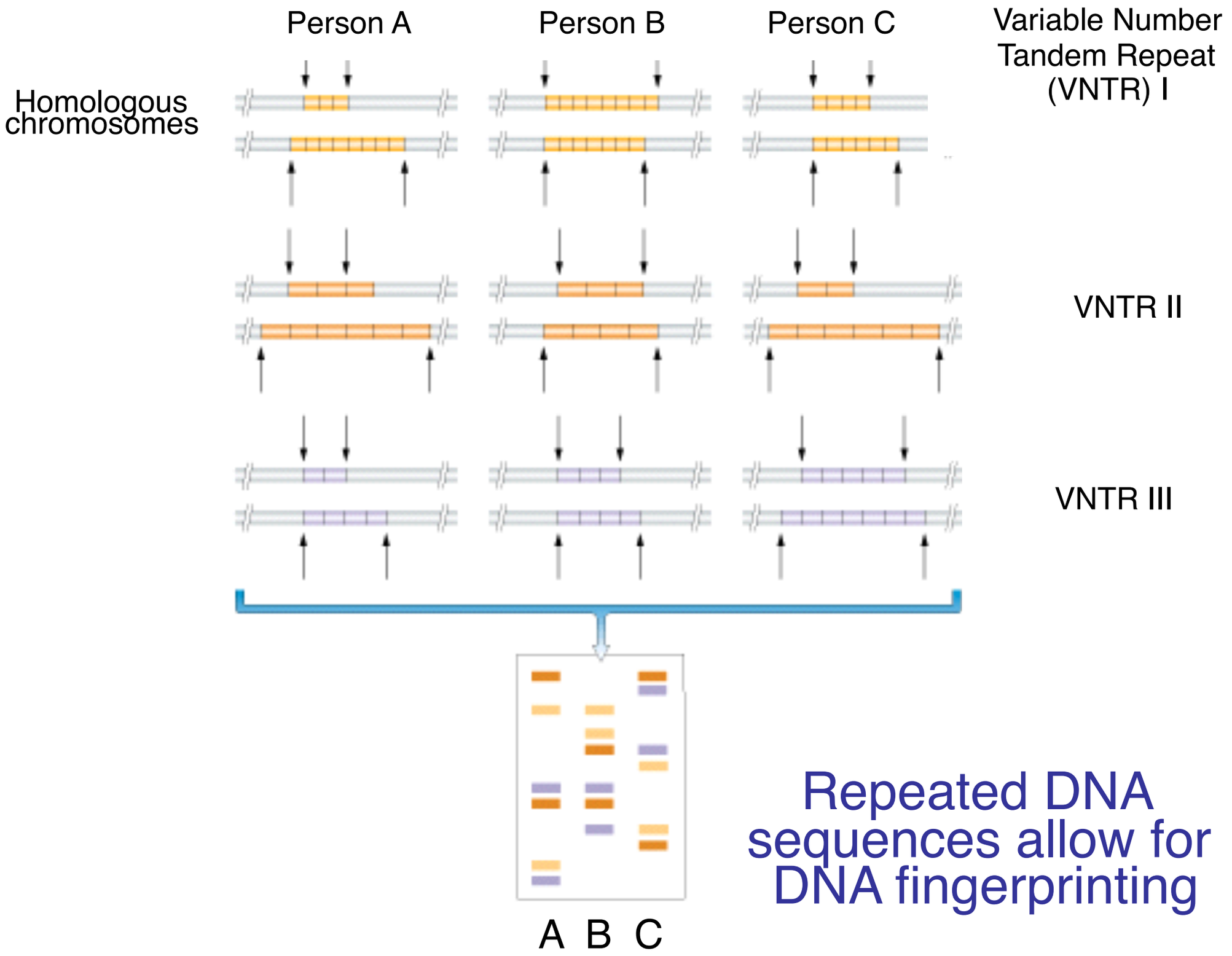
Detection of Trinucleotide Repeat Expansion



Detection of Trinucleotide Repeat Expansion

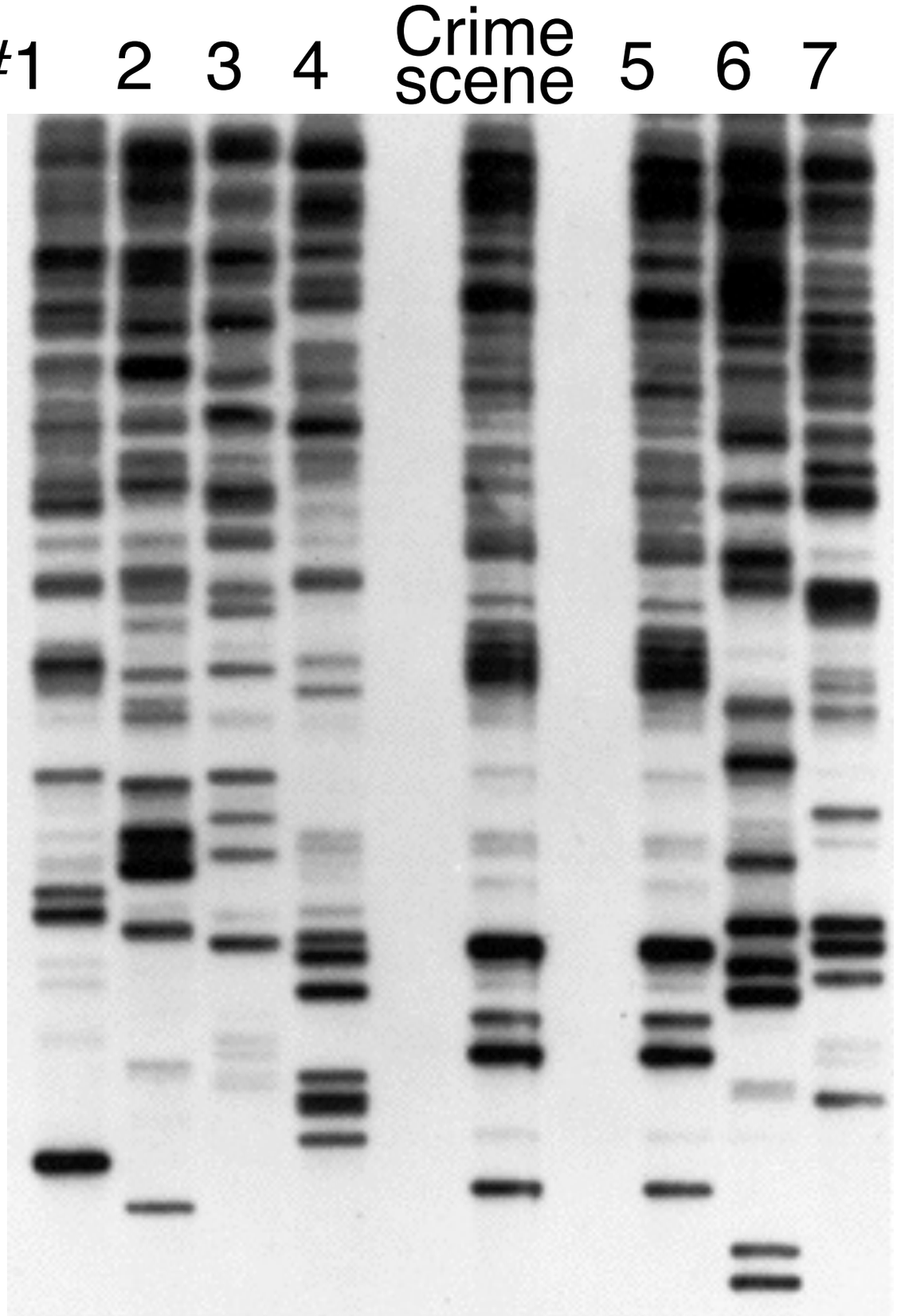


DNA fingerprinting allows for quick comparisons of many loci



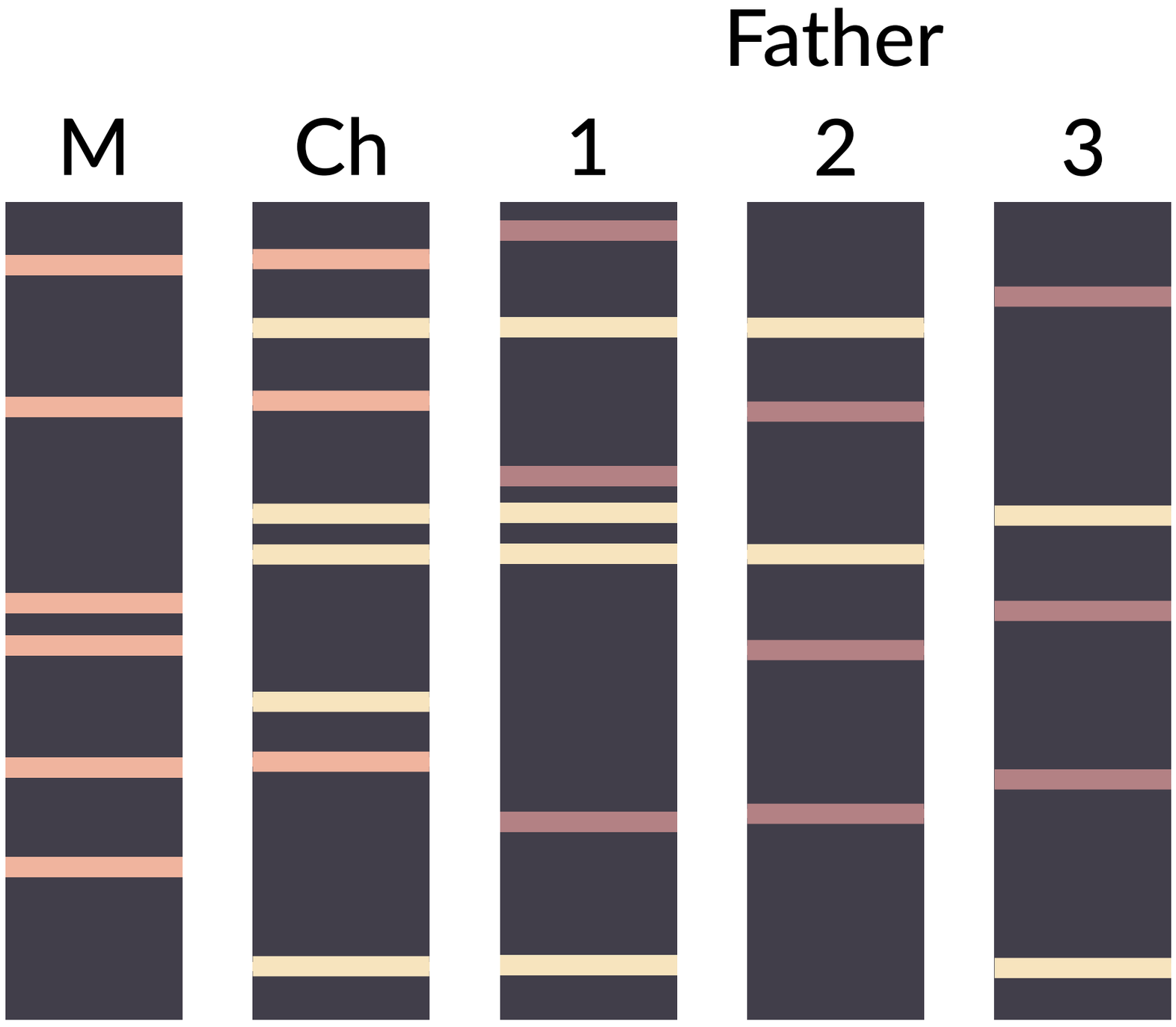
Repeated DNA sequences allow for DNA fingerprinting

Who committed the crime?



Paternity testing & DNA forensics use VNTRs

Who is the father?



Restriction enzymes recognize palindromic DNA sequences (usually)

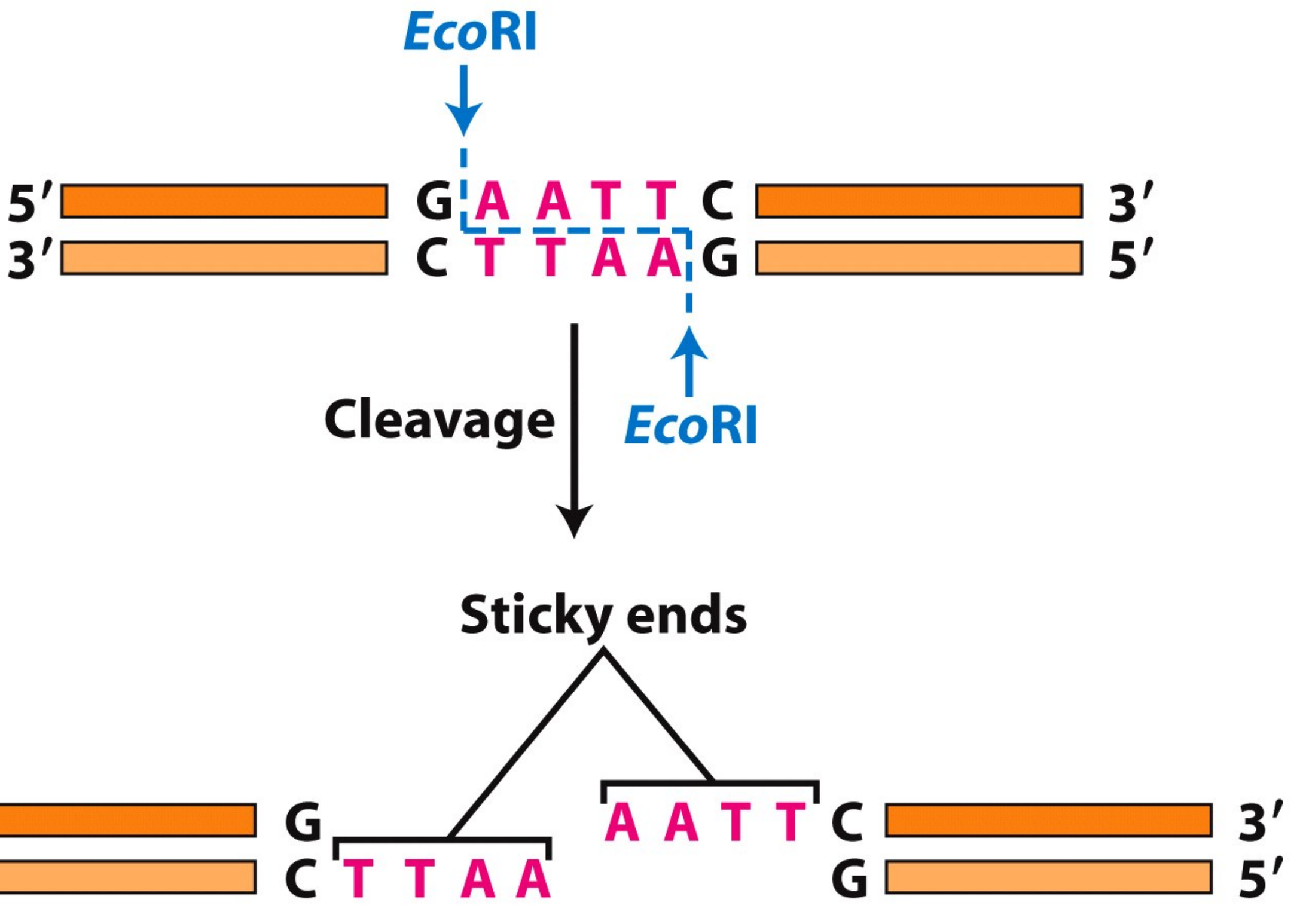
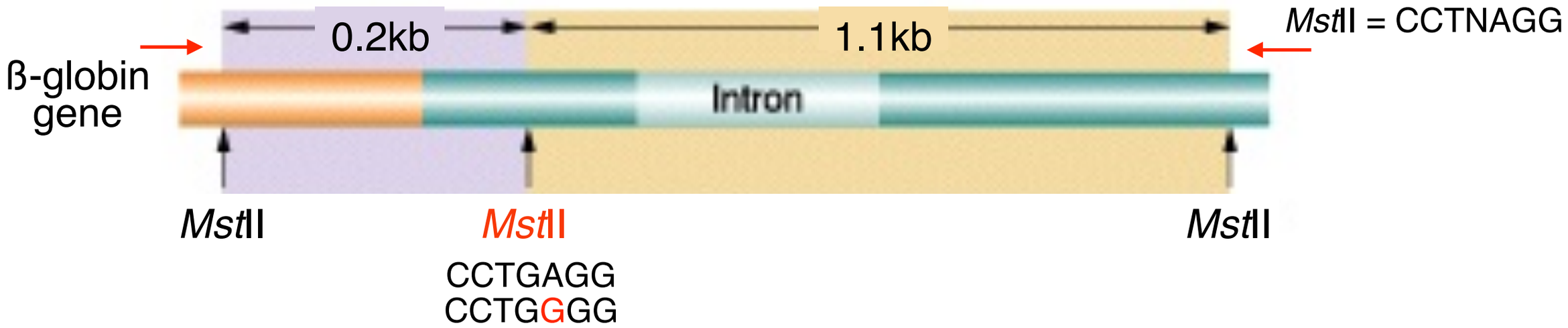


TABLE 5-1 Selected Restriction Enzymes and Their Recognition Sequences

ENZYME	SOURCE MICROORGANISM	RECOGNITION SITE*	ENDS PRODUCED
***Bam*HI**	***Bacillus amyloliquefaciens***	↓ -G-G-A-T-C-C- -C-C-T-A-G-G- ↑	**Sticky**
***Sau*3A**	***Staphylococcus aureus***	↓ -G-A-T-C- -C-T-A-G- ↑	**Sticky**
***Eco*RI**	***Escherichia coli***	↓ -G-A-A-T-T-C- -C-T-T-A-A-G ↑	**Sticky**
***Hin*dIII**	***Haemophilus influenzae***	↓ -A-A-G-C-T-T- -T-T-C-G-A-A- ↑	**Sticky**
***Sma*I**	***Serratia marcescens***	↓ -C-C-C-G-G-G- -G-G-G-C-C-C- ↑	**Blunt**
***Not*I**	***Nocardia otitidis-caviarum***	↓ -G-C-G-G-C-C-G-C- -C-G-C-C-G-G-C-G- ↑	**Sticky**

Diagnostic use of restriction enzymes

Detection of HbS mutation of sickle cell anemia

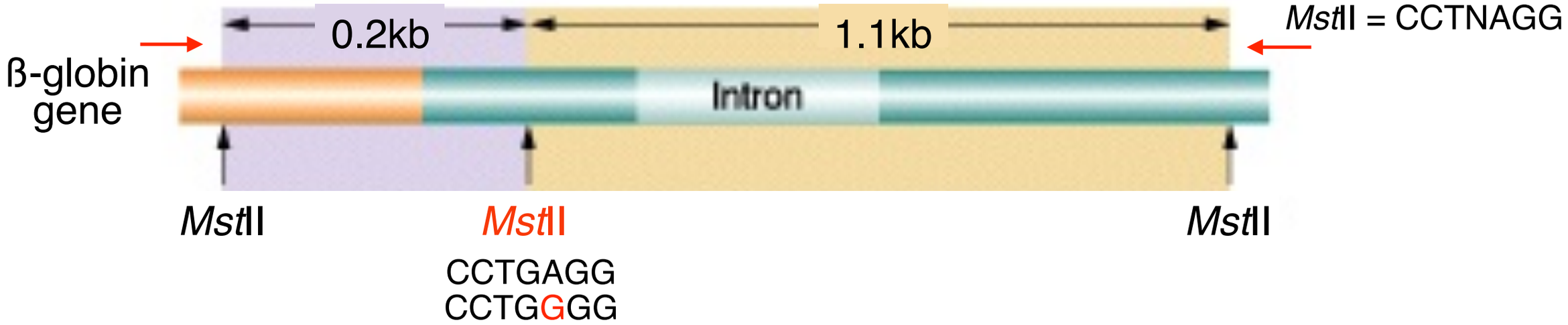


A to G mutation
In HbS destroys *Mst*II site

(example of a **Restriction Fragment Length Polymorphism**; RFLP)

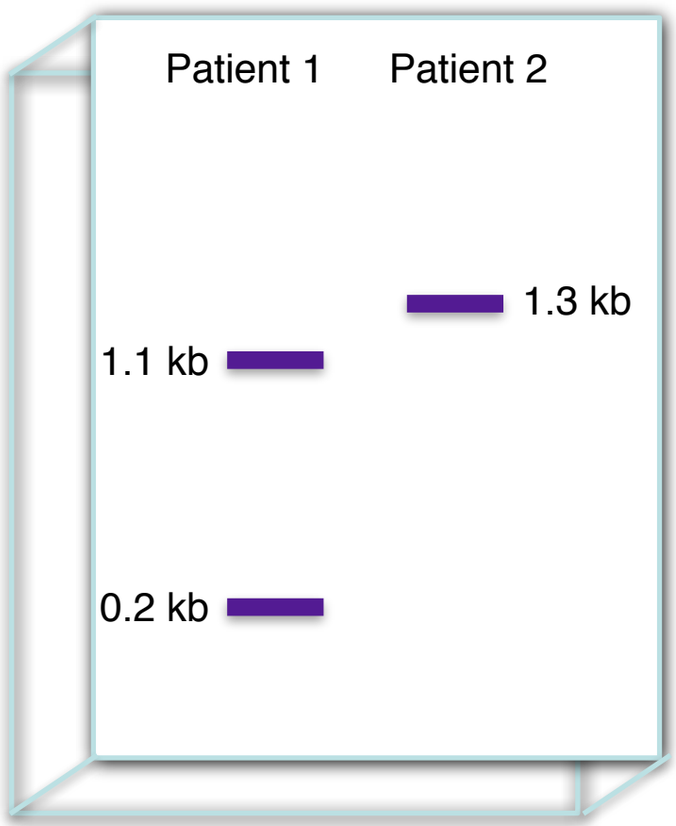
Diagnostic use of restriction enzymes

Detection of HbS mutation of sickle cell anemia



A to G mutation
In HbS destroys *Mst*II site

(example of a **Restriction Fragment Length Polymorphism**; RFLP)

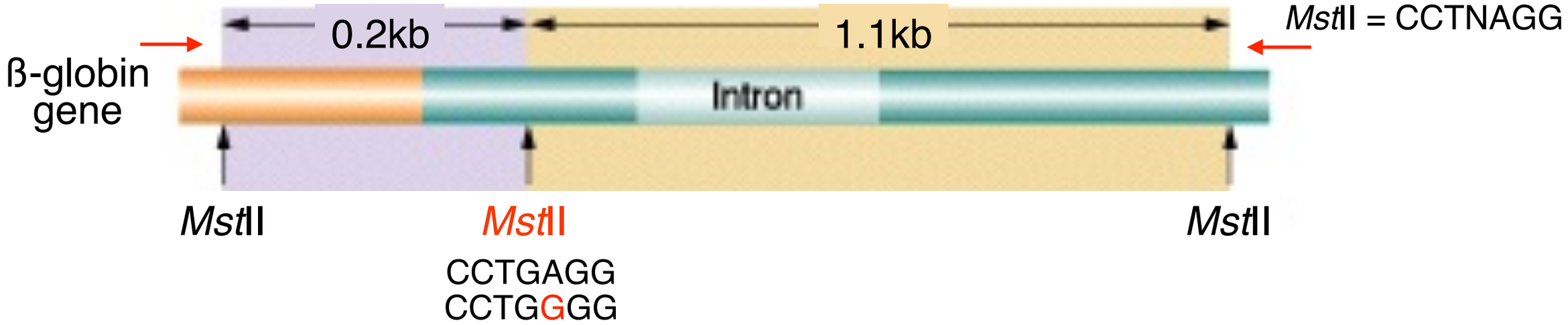


QUIZ: What is the correct interpretation of this result?

A: Patient 1 has sickle cell anemia but Patient 2 does not.
B. Patient 2 has sickle cell anemia but Patient 1 does not.
C. Both patients probably don't have sickle cell anemia.
D. Both patients probably do have sickle cell anemia.

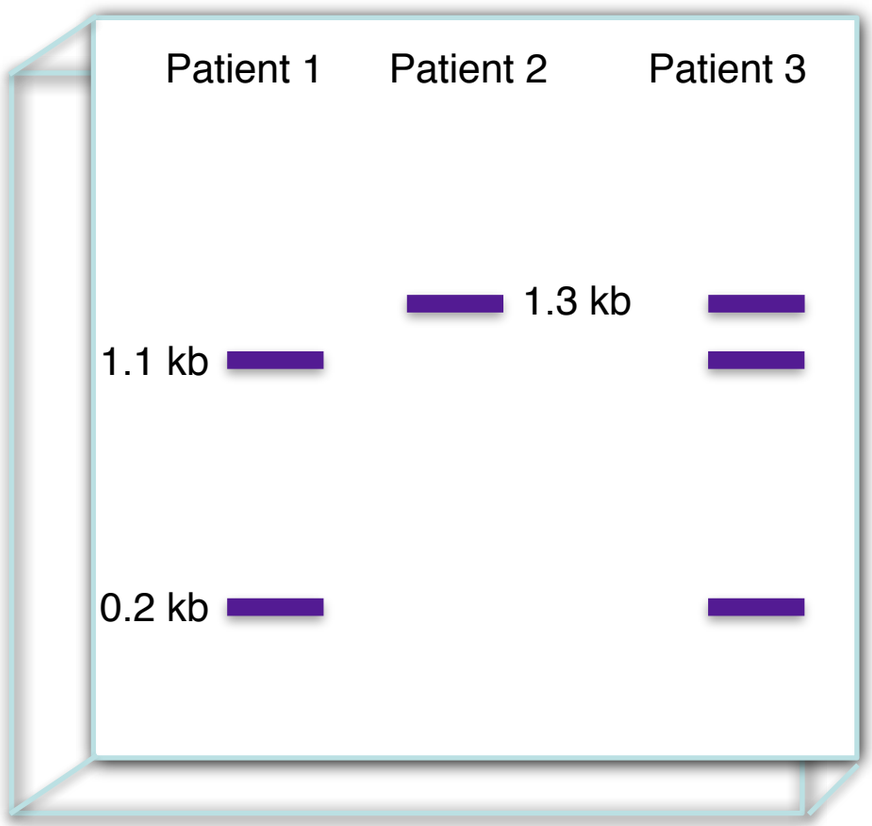
Diagnostic use of restriction enzymes

Detection of HbS mutation of sickle cell anemia



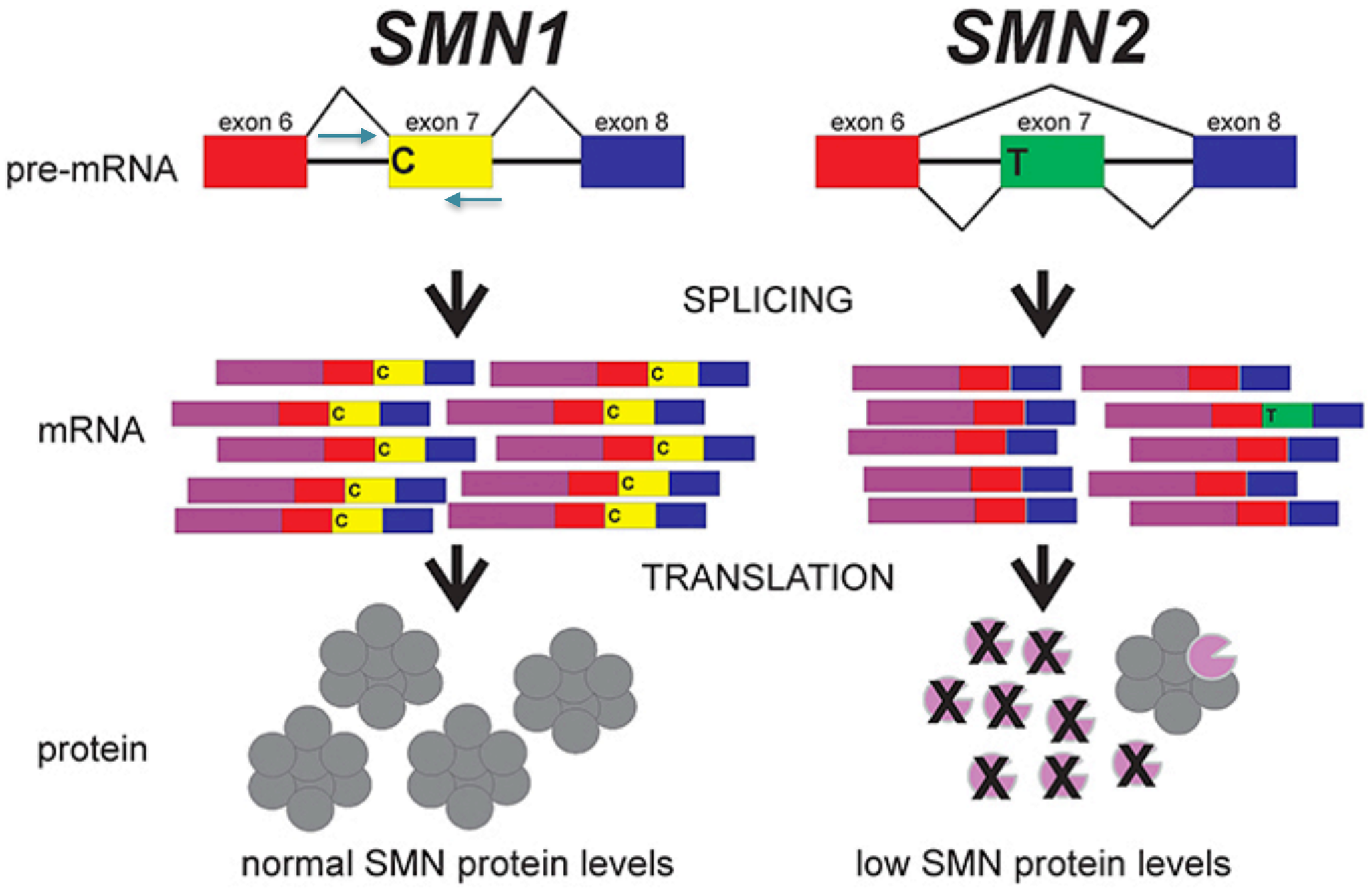
A to G mutation
In HbS destroys *Mst*II site

(example of a **Restriction Fragment Length Polymorphism**; RFLP)



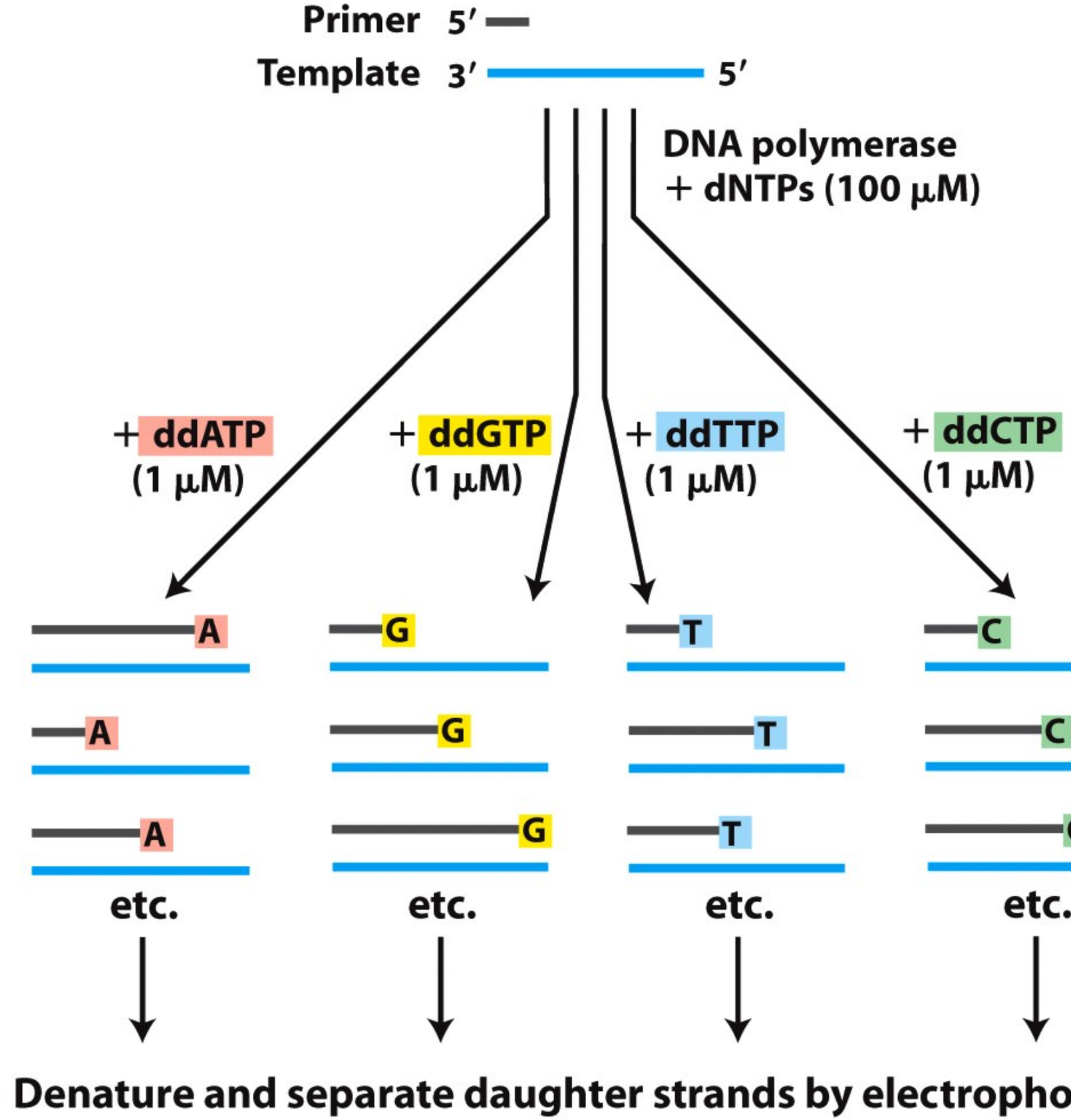
What's going on with patient 3?

What if the mutation isn't within a restriction enzyme site?



Butchbach *Front in Mol Biosci* (2016)

DNA sequencing ("Sanger sequencing")



Key reagent: Dideoxynucleotides

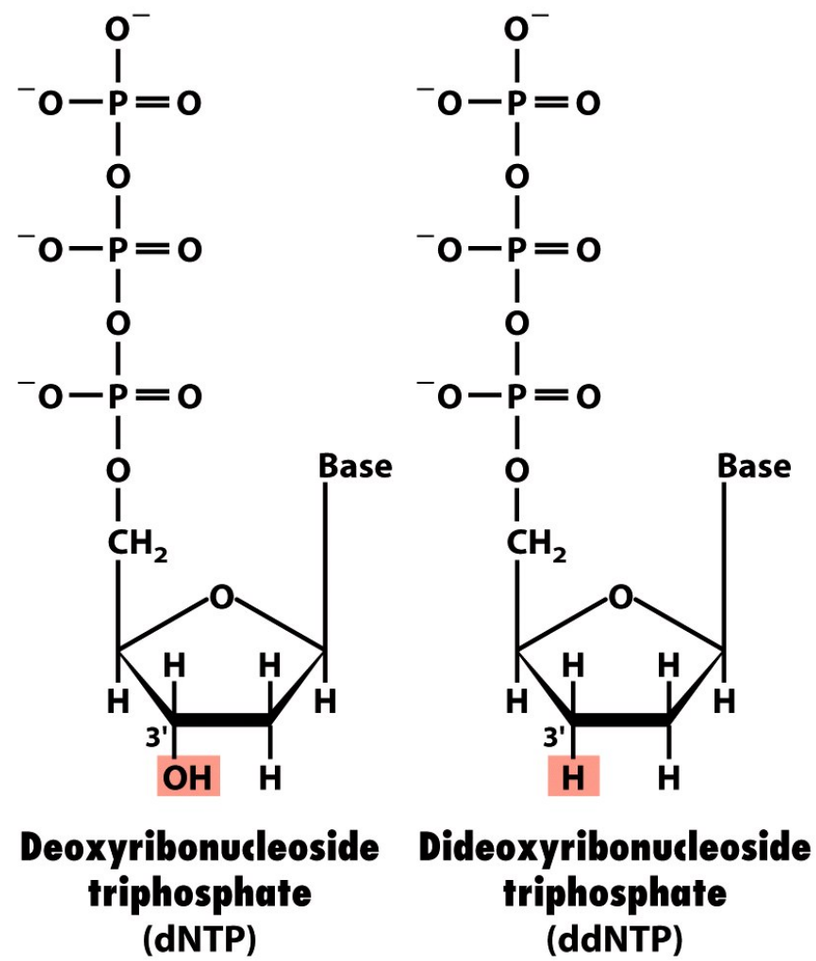
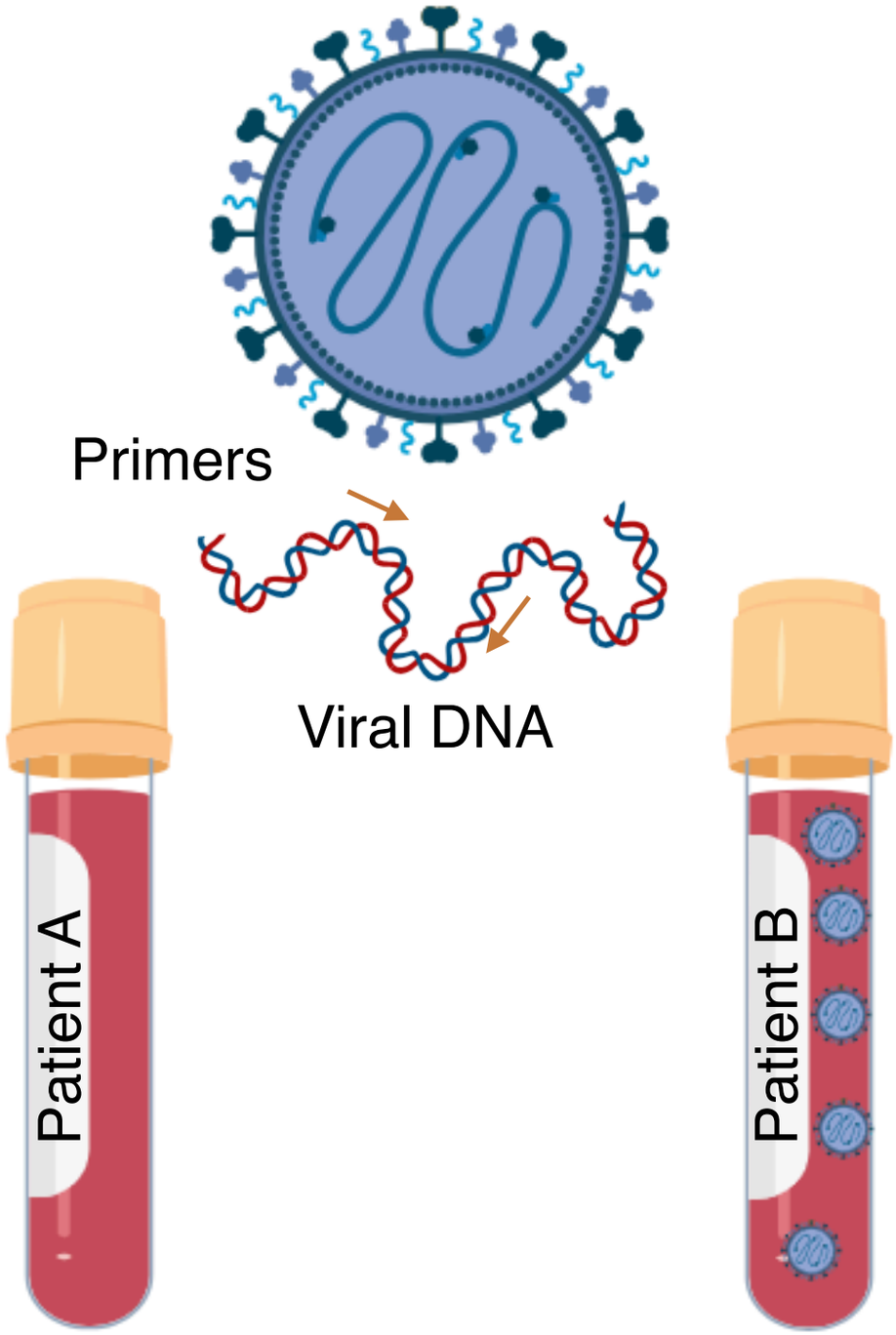
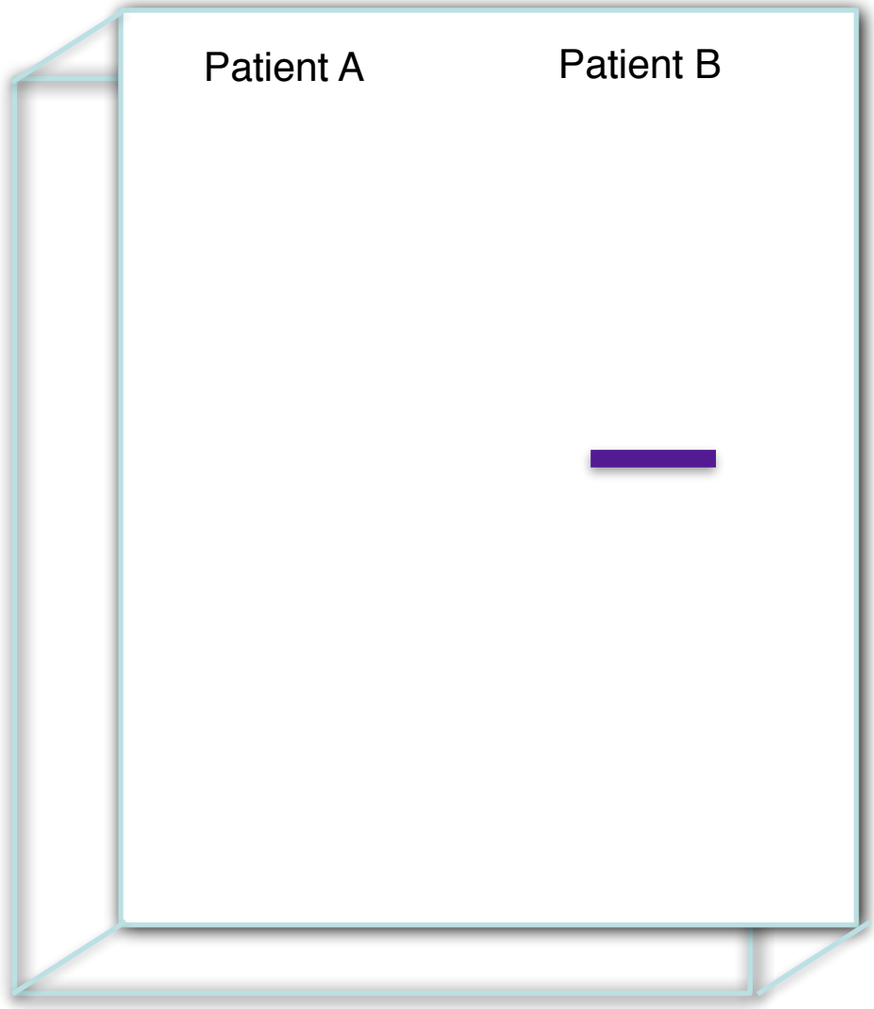


Figure 5-20
Molecular Cell Biology, Sixth Edition

Assaying infection with PCR



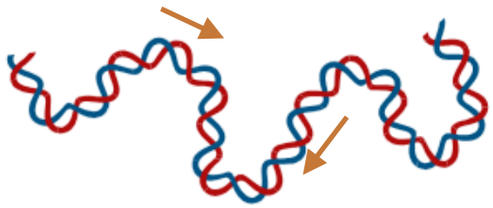


Is there infection or not?
"Yes" or "No" answer

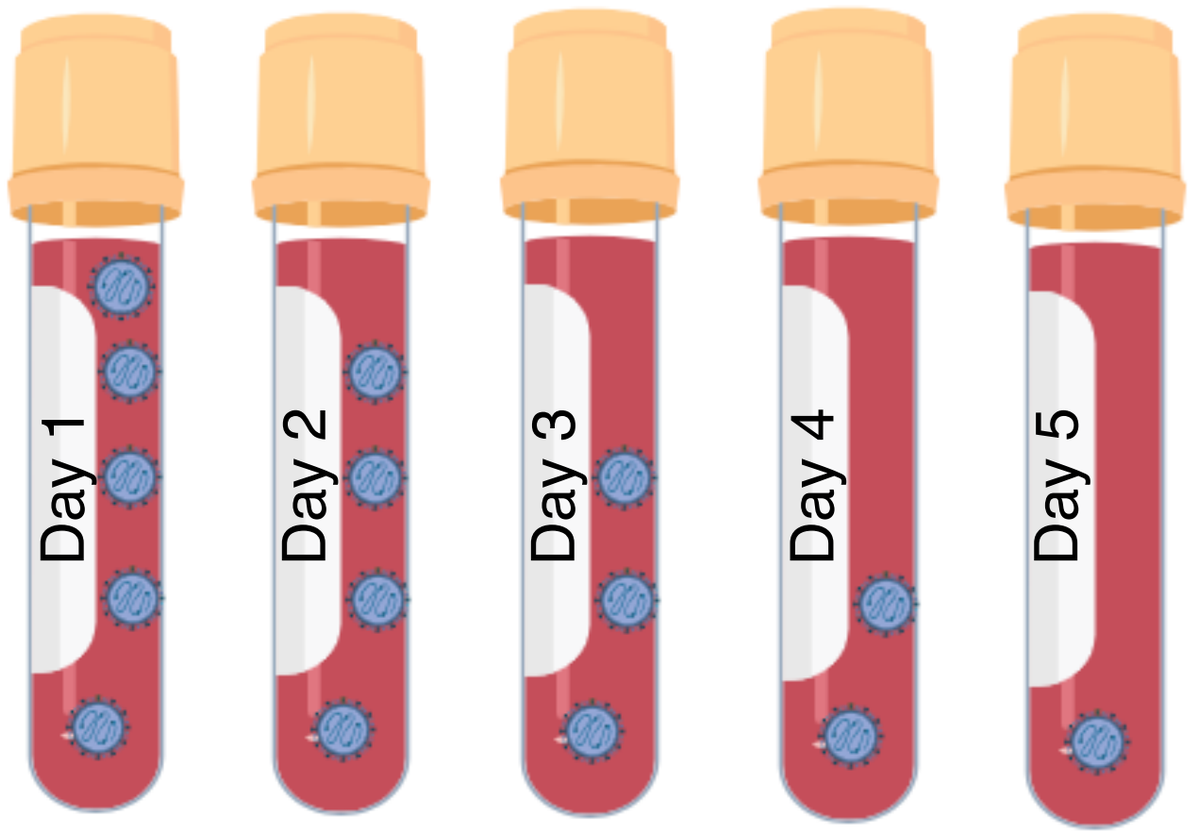
What if we wanted to monitor extent of infection over time?

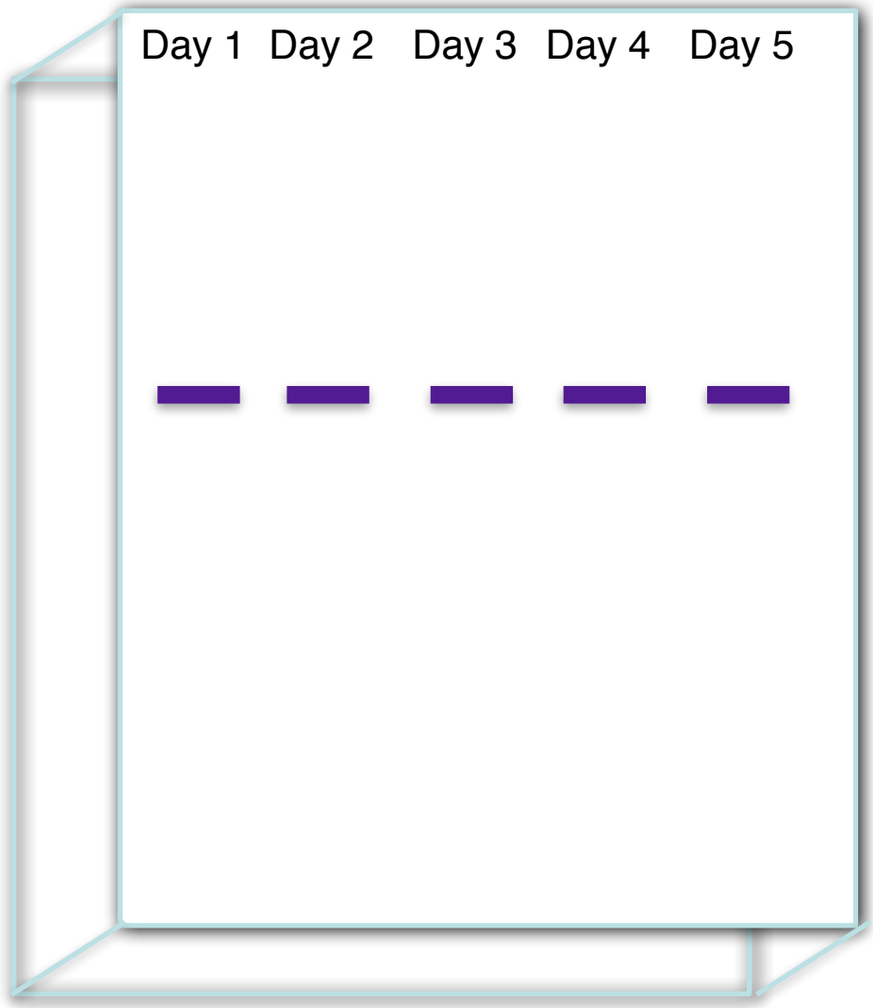
Assaying infection with PCR

Primers



Viral DNA



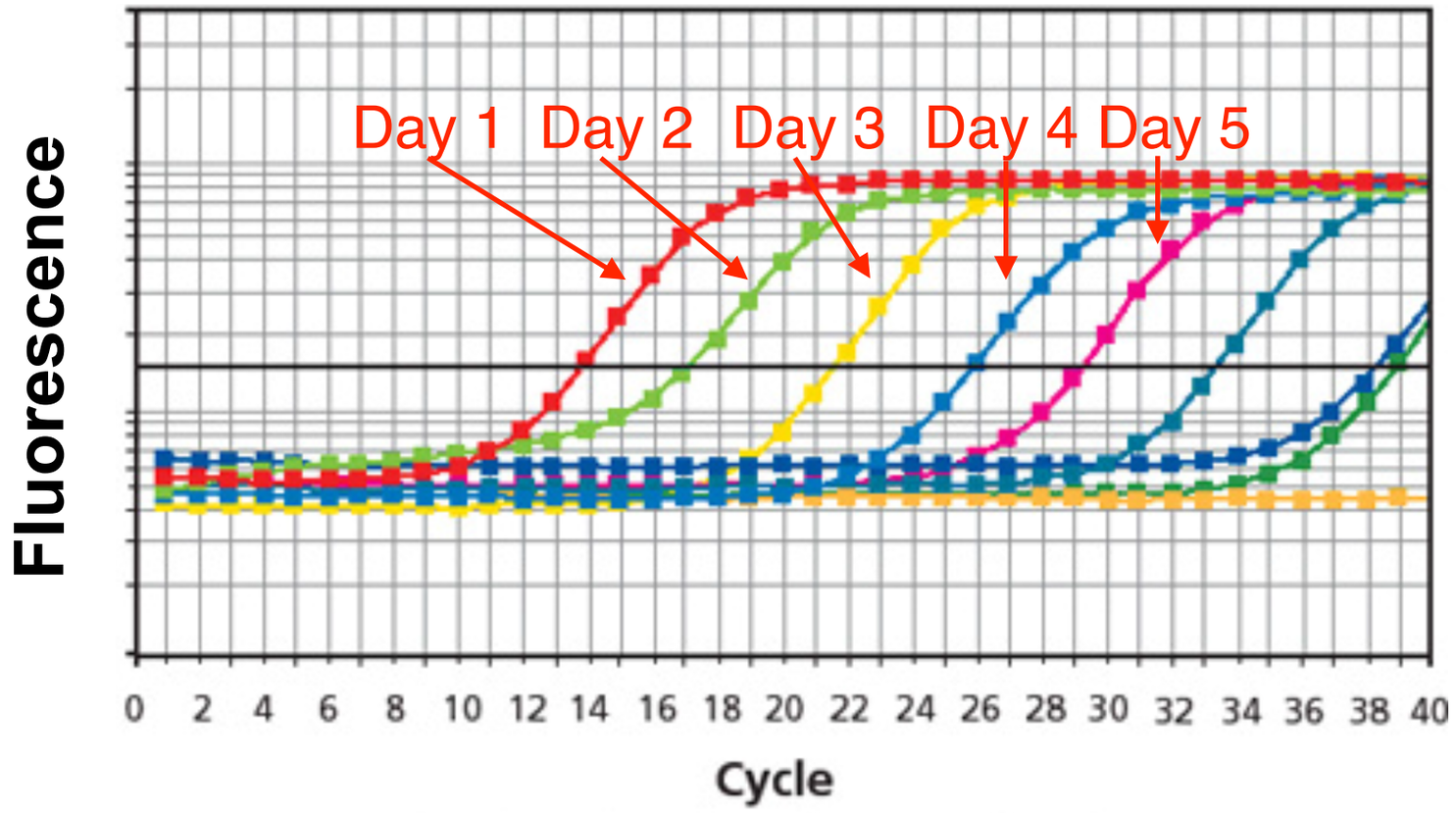


Real-time, quantitative PCR (qPCR) allows estimation of the *abundance* of nucleic acid in a sample

Real-time, quantitative PCR ("qPCR")

1. Dye in solution emits low fluorescence

2. Emission of the fluorescence by binding



Direct readout, so no analysis by gels

MUDDIEST POINT

MUDDIEST POINT

A. Repeat expansion PCR

B. DNA Fingerprinting

C. Restriction fragment length polymorphism

D. Sanger Sequencing

E. Quantitative PCR